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Breaking the Canon: Indirect Regulation of Wnt Signaling in Mammary Stem Cells by MMP3

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Matrix metalloproteases promote tumor cell invasion, epithelial-to-mesenchymal transitions, and metastases, but whether they directly regulate stem cells is unknown. In this issue of *Cell Stem Cell*, Kessenbrock et al. (2013) now show that MMP3, independent of its proteolytic activity, regulates murine mammary stem cells by sequestering noncanonical Wnt signaling ligands, which has implications for breast cancer pathogenesis.

Matrix metalloproteases (MMPs) have numerous functions at the cellular and tissue level, in both physiological and pathophysiological states. MMPs contain protein binding hemopexin (HPX) and transmembrane domains and a catalytic domain that is activated by proteolytic cleavage of a propeptide. Stromelysin-1/MMP3, an MMP expressed mainly in the stroma, is best known for its role in hastening tumor progression by destroying basement membranes via its catalytic domain. Unbridled MMP3 activity causes murine epithelial cells to undergo epithelial-to-mesenchymal transitions (EMTs) and promotes genomic instability (Radisky et al., 2005) at the cellular level, and at the tissue-level, aberrant MMP3 activity in the mammary gland stimulates tumorigenesis (Sternlicht et al., 1999). Mammary gland stem cells express constellations of genes consistent with EMT, a determinant of the stem cell phenotype (Mani et al., 2008), and share other phenotypic similarities with putative breast cancer stem cells, which are thought to drive tumor formation. Tightly controlled canonical Wnt signaling is synonymous with stem cell homeostasis, whereas unregulated Wnt signaling causes mammary tumors in mice (Alexander et al., 2012). Thus MMP activity and Wnt signaling can promote similar biological outcomes and could be functionally intertwined, but evidence supporting this link has been lacking until now. In this issue of *Cell Stem Cell*, Zena Werb and colleagues demonstrate that MMP3 regulates murine mammary stem cell activity through an unexpected mechanism: the noncatalytic HPX domain of MMP3 binds to and inactivates the noncanonical Wnt ligand Wnt5b, resulting in promotion of mammary stem cell activation through the canonical Wnt pathway (Kessenbrock et al., 2013).

Kessenbrock et al. set out to gain a better understanding of the mechanism through which overexpression of MMP3 in transgenic models induced mammary tumors (Sternlicht et al., 1999). To do so, they utilized lentiviruses to transduce primary mammary epithelial cells (MECs) with secreted versions of a full length furin-activateable MMP3 (MMP3-FL), a proteolytically inactive mutant, or the MMP3-HPX domain by itself. Transduced MECs were then implanted into cleared mammary fat pads, where mammary epithelia can undergo extensive morphogenesis. MECs potentially give rise to functional mammary glands or to various stages of pathology depending on the genotype of both the implanted cells and the stroma. MMP3-FL transduced MECs gave rise to hyperbranching epithelial outgrowths, consistent with previous reports in MMP3 transgenic mice (Sternlicht et al., 1999). Surprisingly, both the proteolytically inactive mutant and the HPX domain alone gave rise to an identical hyperbranching phenotype. The authors further showed that the HPX domain of MMP10, though highly homologous to the MMP3 HPX, had no effect on gland morphology, thus demonstrating a specialized function of the MMP3-HPX domain.

Kessenbrock et al. then identified binding targets of MMP3-HPX that could explain this outgrowth phenotype. Yeast two-hybrid analysis using MMP3-HPX as bait identified 21 proteins, including the extracellular noncanonical Wnt ligand Wnt5b, as potential candidates. The MMP3-HPX/Wnt5b interaction was specific because HPX domains from mammary MMPs 2, 10, and 14 did not interact with Wnt5b, and MMP3-HPX did not interact with other mammary Wnts 2, 4, 6, 7b, or 10b. Using a version of Wnt5b with tagged C and N termini, the authors showed that Wnt5b is a bona fide cleavage substrate of MMP3, as well as a binding target of the HPX domain. Thus MMP3 dually antagonizes Wnt5b by interfering with receptor binding and by irreversible proteolysis. This two-pronged inhibitory approach requires relatively few molecules of MMP3 in order to antagonize Wnt5b.

Kessenbrock et al. next assessed the phenotypic consequences of the MMP3/Wnt5b interaction and how Wnt5b might mediate these effects. Overexpression of Wnt5b in MECs suppressed branching and mammary epithelial outgrowths. Wnts are known to signal either in a canonical fashion that results in nuclear localization of β-catenin or in a noncanonical fashion via nuclear factor of activated T cells (NFAT) (Alexander et al., 2012). Wnt5b overexpression caused the accumulation of NFAT in MECs, consistent with noncanonical signaling, which was antagonized by addition of MMP3-FL. Conversely, either MMP3-FL or Wnt1 overexpression caused the hyperbranching phenotype, which was accompanied by a striking increase in nuclear β-catenin and expression of the Wnt responsive gene Axin2, consistent with activation of canonical Wnt signaling. Thus when MMP3 activity was high, Wnt5b was inactivated and the canonical Wnt pathway was activated by one of the several mammary-resident Wnts. When MMP3 was not overexpressed, intact Wnt5b

was able to suppress the canonical pathway. The exact mechanism by which Wnt5b suppresses the canonical pathway is unknown, but may involve competition for the frizzled coreceptors (Grumolato et al., 2010).

The authors then provided direct evidence that MMP3 modulates mammary stem cell activity. They showed that MMP3-overexpressing MECs had an expanded CD24/CD49f^{hi} stem-cell-enriched fraction, and they produced more mammospheres in culture compared to control MECs. Increased mammosphere production from MMP3-FL MECs was prevented by addition of the tankyrase inhibitor JW-55, which blocks canonical Wnt signaling. Conversely, MMP3 null mice exhibited decreased numbers of stem cells and produced half as many mammospheres. Both of these phenotypes were reversed by addition of canonical Wnt3a. The authors performed mammary fat pad reconstitution assays to demonstrate that regenerative capacity of mammary epithelia from MMP3 null mice was reduced 7-fold compared to epithelia from control mice. Thus Kessenbrock et al. provide an elegant mechanism for control of mammary stem cells in mice, showing that MMP3 sequesters Wnt5b and promotes stem cell activity by allowing canonical Wnt signaling to proceed (Kessenbrock et al., 2013). Accordingly, the patterns of MMP3 and Wnt5b expression in mammary gland are complementary to known developmental stage-specific stem cell requirements: MMP3 levels increase from early to midpregnancy as the epithelia expands as much as 10-fold to prepare for lactation, then completely disappears as Wnt5b reaches its highest levels just before lactation begins (Gavin and McMahon, 1992; Sorrell et al., 2005). MMP3 also is highly expressed during mammary gland involution, which is perplexing in the present context and suggests that mechanisms exist to compartmentalize MMP3 activity into either roles that regulate mammary stem cell behavior or roles that play a purely proteolytic function.

Even in normal, nontransformed MECs, the principal phenotypes reported in conjunction with overexpressed MMP3 or canonical Wnts were consistent with abnormal hyperplastic pathologies. Another study recently demonstrated that MMP3-HPX binds to a secreted form of HSP90β, causing the hyperbranching morphology to emerge in MECs embedded in organotypic 3D cultures (Correia et al., 2013). Beyond Wnt5b, Kessenbrock et al. and others identified a number of MMP3-HPX targets that will need to be studied in greater detail for us to fully understand the extent of this molecule's functional pleiotropy. When viewed from a broader perspective, these studies may help connect changes in the stromal microenvironment to behavior that is often associated with cancer stem cell activity. Tumor microenvironments are known to possess numerous factors capable of inducing EMT gene programs, such as MMP3, which could possibly impose cancer stem-cell-like states (LaBarge, 2010). Thus MMPs may be the perfect fuel for tumor progression because they not only destroy the basement membrane responsible for providing polarity cues, but they also may indirectly drive the stem-cell-like states thought to be synonymous with invasion and metastasis.

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